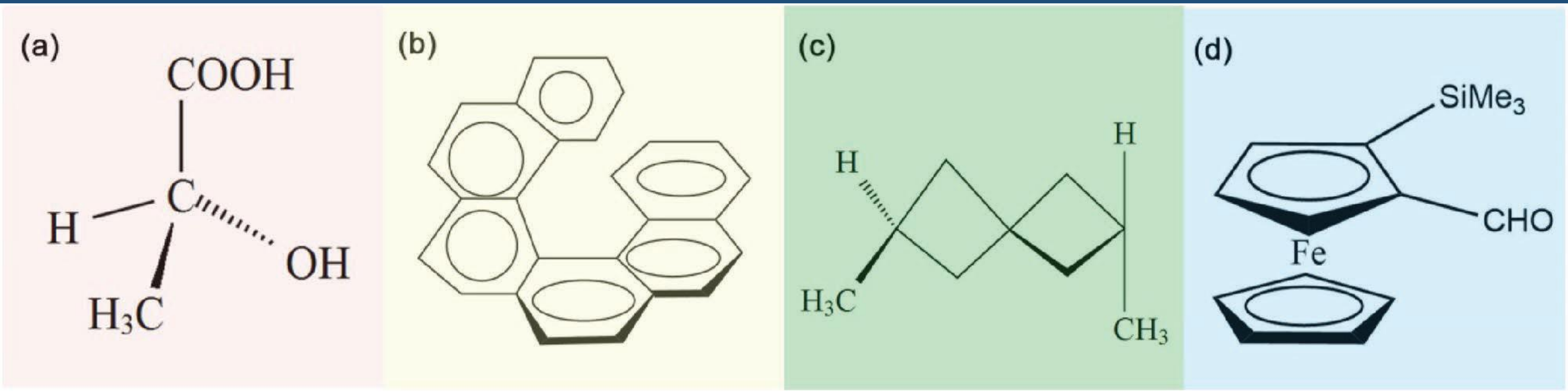
Circular Dichroism Spectroscopy



a) Central chiral molecule. b) Helical/spiral chiral molecule. c) Axial chiral molecule. d) Planar chiral molecule

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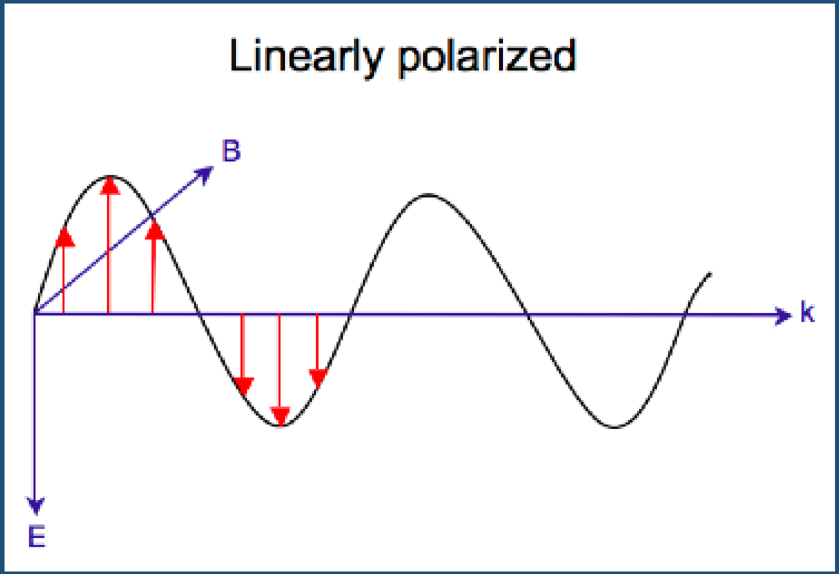
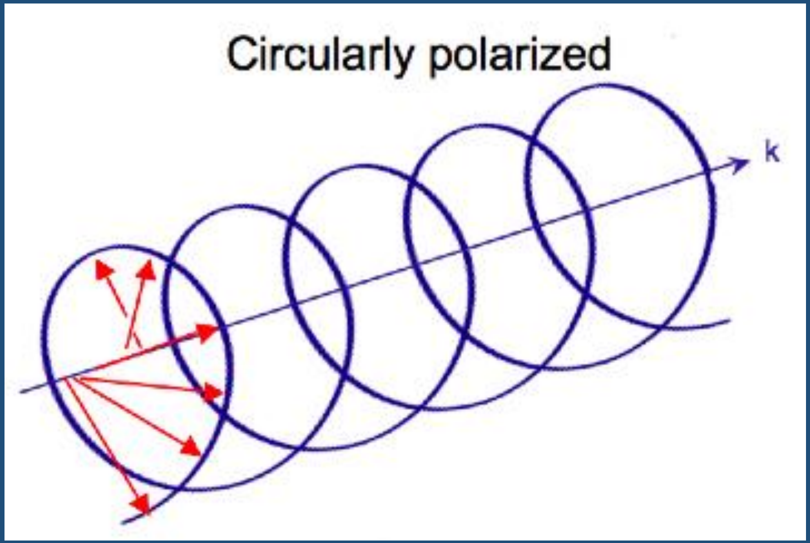
Origin of optical rotation and circular dichroism

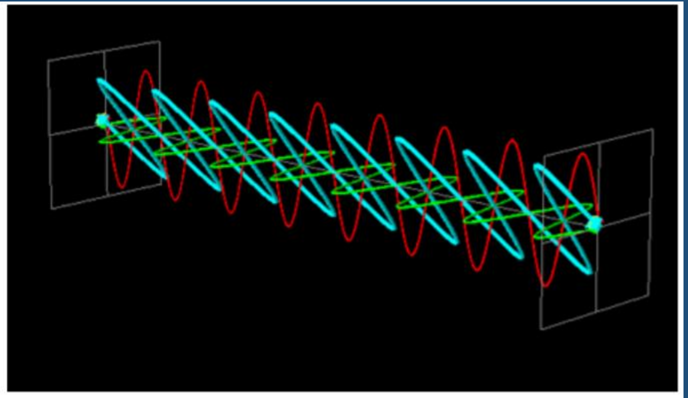




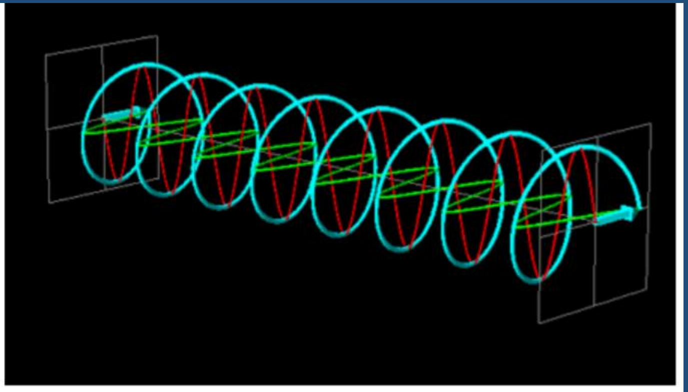
- Electromagnetic radiation consists of an electric (E) and magnetic (B) field that oscillate perpendicular to one another and to the propagating direction.
- Linearly polarized light = when the electric field vector oscillates only in one plane,
- Circularly polarized light = when the direction of the electric field vector rotates about its propagation direction.
- In linear polarized light the direction of the vector stays constant and the magnitude oscillates. In circularly polarized light the magnitude stays constant while the direction oscillates.

Origin of optical rotation and circular dichroism

- If we take horizontally and vertically polarised light waves of equal amplitude that are in phase with each other, the resultant light wave (blue) is linearly polarised at 45 degrees.
- If one of the polarised states is out of phase with the other by a quarter-wave, the resultant will be a helix and is known as circularly polarised light (CPL). The helices can be either right-handed (R-CPL) or left-handed (L-CPL) and are non-superimposable mirror images.
- If the vector rotates counterclockwise when the observer looks down the axis of propagation, the light is left circularly polarized (LCP).
- If it rotates clockwise, it is right circularly polarized (RCP).
- Measuring optical rotation as a function of wavelength is termed optical rotatory dispersion (ORD) spectroscopy.
- Left-and right-circularly polarised light will be absorbed to different extents by a chiral medium. This difference in absorption of L-CPL and R-CPL is called circular dichroism.



45 Degree Polarised Light



Left Circularly Polarised (LCP) Light

Interaction with matter

As with linear polarized light, circularly polarized light can be absorbed by a medium.

Circular dichroism is defined as, $\Delta A = A_l - A_r$

Extending this to Beer-Lambert Law, $\Delta A = (\varepsilon_l - \varepsilon_r)cl$

The difference in molar absorptivity is also known as the molar circular dichroism,

$$\Delta\varepsilon = (\varepsilon_l - \varepsilon_r)$$

The CD spectrum is often reported in degrees of ellipticity, θ, which is a measure of the ellipticity of the polarization given by:

$$tan\theta = \frac{E_l - E_r}{E_l + E_r}$$

where E is the magnitude of the electric field vector.

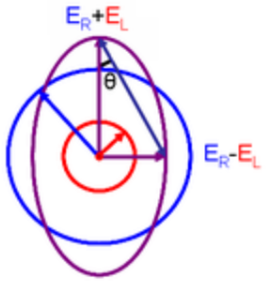
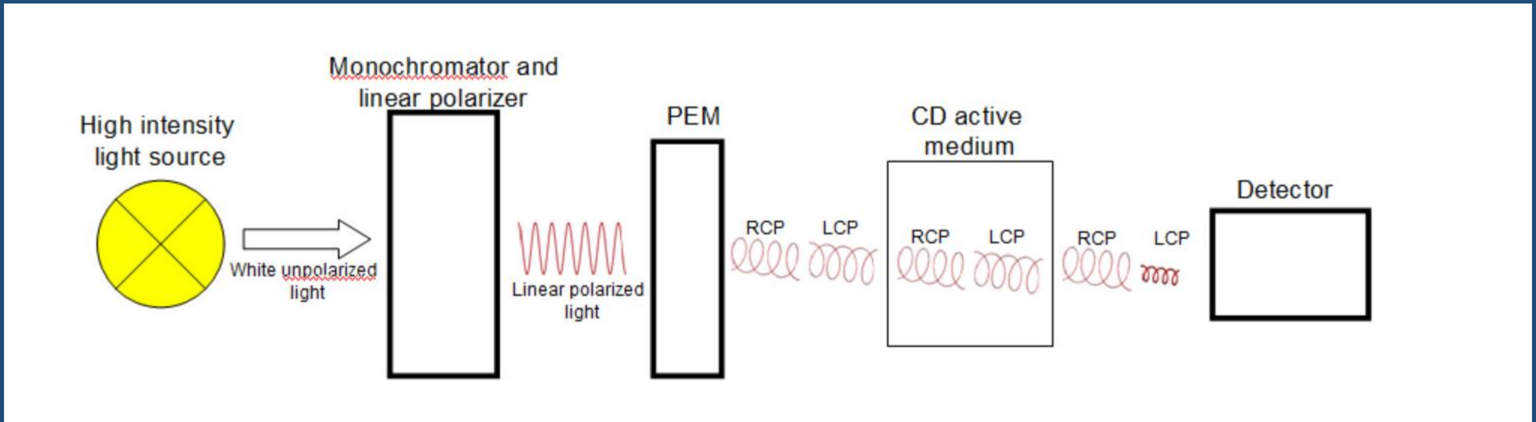


Figure 4: Elliptically polarized light (purple) is the superposition of LCP (red) and RCP (blue) light. θ is the angle between the magnitude of the electric field vector at its maximum and its minimum

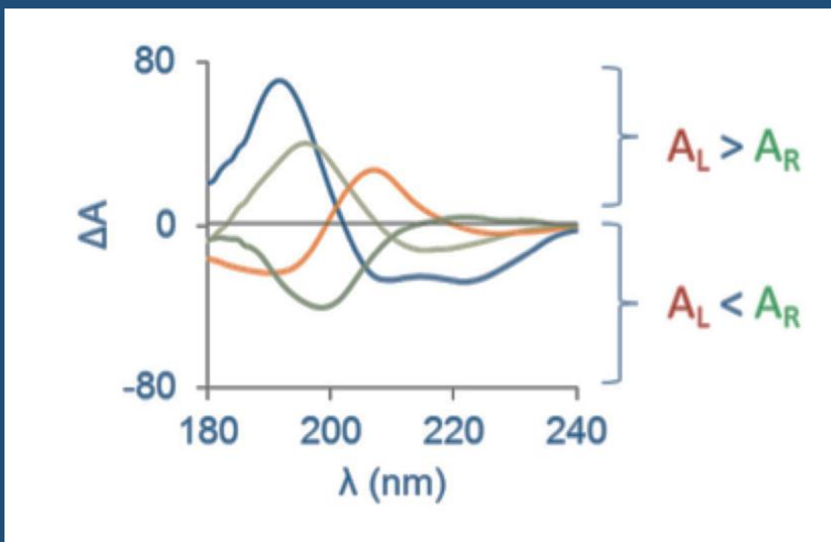
CD Spectrometer: Working principle

- Most commercial CD instruments are based on the modulation techniques introduced by Grosjean and Legrand.
- Light is linearly polarized and passed through a monochromator.
- The single wavelength light is then passed through a modulating device, usually a photoelastic modulator (PEM), which transforms the linear light to circular polarized light.
- The incident light on the sample switches between LCP and RCP light. As the incident light switches direction of polarization the absorption changes and the difference in molar absorptivity can be calculated.



The instrumentation for a common CD spectrometer showing the polarization of light and the differential absorption of LCP and RCP light.

- When L-CPL is absorbed to a greater extent than R-CPL, CD signal is positive.
- When L-CPL is absorbed to a lesser extent than R-CPL, CD signal is negative.
- Chirascan circular dichroism spectrometers measure alternately the absorbance of L- and R-CPL and then calculate the CD signal.
- A CD spectrum may have both positive and negative CD intensity.
- Enantiomers show CD spectra of mirror image relationship.



Circular dichroism spectra vary according to differences in absorbance of
L- and R-CPL.

Application and Advantages

1. Extensively useful to determine secondary structures of proteins and peptides.
2. Useful to determine structural and thermodynamic properties.
3. (i) spectra can be recorded in minutes and (ii) single wavelength kinetics can be recorded from milliseconds onwards, making CD a particularly powerful tool to follow dynamic changes in protein structure.
4. Changes induced by changing temperature, pH, ligands, or denaturants are all commonly used.
5. Folding and unfolding mechanisms of proteins can be studied.
6. Chiral nanoparticles, helically assembled nanostructures also can be studied.

Thank you!